Effect of Nitrogen Source on Ureides in Soybean

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ABSTRACT

In field-grown soybeans (Glycine max L. Merr. cv Harosoy), the percentage of N in the xylem as ureides increased with increasing N2 fixation. During a 9-week collection period, the ureide content varied from 9.0 to 69.2% of the xylary N. Between 9 and 11 weeks (early pod fill), there was a good correlation (r = 0.93) between C2H2 reduction and the per cent N in xylary ureides. The per cent N as ureides, however, does not always indicate the reliance of the plant on symbiotic N2 fixation. This ureide content also depended on the level of NO3- available to the roots. Non-nodulated soybeans given 0 to 200 kilogram N per hectar produced xylem sap which averaged from 31.8% to 9.0% N, respectively, in the xylem as ureides over the 9-week period.

Feeding of 15N2, 15NH4, or 15NO3 to greenhouse-grown soybeans indicated substantial differences in the initial distribution of N by the xylem stream, but the ultimate distribution of N between plant parts and grain did not vary with available N or percentage of xylary N as ureides. Amino acids, not ureides, were the major source of N in the phloem. The soybeans maintained a similar composition in phloem irrespective of the xylary products, with N derived from N2, NH4, or NO3 being equally accessible to the phloem stream.

In the xylem of greenhouse-grown soybeans, there are differences between xylary products of N2 fixation and nitrate reduction (14, 15, 27). The products of N2 fixation are mostly ureides (allantoin and allantonic acid), whereas amino acids (predominantly asparagine) and NO3- result from NO3- uptake. Ureides were observed in the xylem sap of field-grown soybeans (26) and were the dominant N compound after 40 d growth, but the relationship between ureides and N2 fixation or soil N was not determined. During early pod fill, the amount of ureides extracted from shoot tissue of field-grown soybeans is significantly correlated with nitrogenase (C2H2) activity (20). Herridge (9, 10) has proposed that the relative ureide content (ureide N/ureide + nitrate N) in soybean tissue can be used to estimate the amount of fixation in the field.

Techniques are now available for collecting phloem sap from soybeans (7, 12) but few studies (11, 13) have determined its composition. Little is known of how the N content of phloem may vary with growth conditions or plant development. We have examined the transport products of N incorporation in xylem and phloem under conditions in which N uptake by soybeans was varied. The consequent partitioning of the N within the plant was determined and compared to differences in the individual constituents of the xylem and phloem to characterize the response of the plant to changes in its N source.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Nodulating and non-nodulating soybean isolines (Glycine max L. Merr. cv 'Harosoy') were grown in a greenhouse with supplemental lighting (Multi vapor, 450 μE m-2 s-1 in a 14:10 h, 27°C:24°C, light:dark regime). Plants were grown two/6-L pot in coarse silica sand, inoculated with Nitravig Rhizobium japonicum inoculant, watered twice daily, and given 100 ml of N free nutrient solution (6) three times weekly. The non-nod3 plants were treated similarly, except the nutrient solution contained either 40 mm KNO3 or 20 mm (NH4)2SO4.

At weekly harvests, petiole phloem sap and xylem sap were collected. Plants were divided into roots, nodules, stems plus petioles, leaves, and flowers, and pods and seeds. Harvests were either four plants or triplicate samples of four plants.

Field-grown plants were sown at Aurora, NY in blocks of four rows, 6 m long × 35 cm apart, at a density of 16 plants/m of row. The center two rows were sampled. The field had previously been sown with corn and, without supplemental N, produced poor growth of non-nod soybeans. We have described estimates of N2 fixation in immediately adjacent plots (19). P and K were applied to the field at recommended rates before planting. Weeds were controlled by Treflan and hand cultivation. Potassium nitrate was applied to the surface of some of the plots (four blocks per treatment) at weeks 1 and 3 after planting (at total rates of 20 or 100 kg N/ha). Some plots received additional applications of KNO3 at 20 or 100 kg N/ha at week 8. Plants were harvested weekly from 5 to 13 weeks after planting and at maturity. Ten plants were collected at each harvest, and triplicate samples of 10 plants were taken at the last harvest.

15N Experiments. Greenhouse-grown non-nod plants were given either 200 ml of 99% enriched (15NH4)2SO4 (5 mmol), or K15NO3 (10 mmol) at 54, 64, and 74 DAP. At the same time, nodulated plants were fed 15N-enriched N2 from which NO had been removed (3). Pots of intact nodulated plants were enclosed in plastic bags which were sealed around the plant stems. The enclosed gas was replaced by a mixture of Ar:O2 (3:1) which subsequently had 15N-enriched N2 added to produce a final gas mixture at harvest of approximately 20% (v/v) O2; 1% 2% (v/v) CO2; 50% (v/v) Ar; and 30% (v/v) N2 (approximately 20 to 30% enriched with 13N). Gas samples were analyzed separately for each pot at the beginning and end of each feeding period on a Micromass 622 mass spectrometer. At either 1.5 or 4 h after application of the 15N sources, the pots were thoroughly leached with nutrient solution and water to remove the label. Samples of four plants were harvested at 1.5 h and 7, and 14 d after application of the 15N. The enrichment with 15N was determined on N2 gas produced from Kjeldahl digests (21) with a mass spectrometer. Phloem sap and xylem sap samples were collected

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3 Abbreviations: non-nod, non-nodulating; DAP, days after planting.
from some plants before the leaching of the label, and the acidic, basic, and neutral components were separated by ion exchange resins (1) from the xylem fluid. All fluids were analyzed for sugars, nitrogenous constituents, and $^{15}$N enrichment.

**Xylem Sap Collections.** Xylem exudate was collected for 1 h from cut stems after the surrounding soil had been thoroughly watered. Collections were made from 10 to 15 plants. The pooled sap was frozen and stored for later analysis.

**Phloem Sap Collection.** Phloem sap was collected from cut petioles or attached pods in 20 mm EDTA, 5 mm KCl, pH 7.0 solutions as described by King and Zeevaart (12) and Fellows et al. (7). Collections were made for 2 h after cutting. The excised petioles and attached leaflets were placed in the dark and kept at approximately 20°C inside plastic containers to reduce transpiration. Pods remained attached to the plant for the entire collection period. Each sample was the bulked exudate of 6 to 12 petioles or 4 to 6 pods, with triplicate samples taken on several occasions.

**Analytical Techniques.** Total amino acids were estimated by ninhydrin analysis (17). Total N was determined by Kjeldahl analysis with the salicylic acid modification to include NO$_3$N (2). Nitrate was determined by the nitration of salicylic acid in concentrated H$_2$SO$_4$ (4). Total sugars in phloem sap were measured using the reaction with phenol in H$_2$SO$_4$ (5), and reducing sugars by the copper reduction technique (25). Ureides were measured using differential hydrolysis followed by colorimetric estimation of the glyoxylic acid produced (28). Because the sum of the NO$_3$, ureides, and amino acids accounted for over 90% of the total N determined by Kjeldahl, this sum was used for calculating the percentage composition of the sap samples.

Nitrogenase (C$_2$H$_2$) was assayed on triplicate samples of two roots from which the soil was lightly shaken. The roots were placed in 1-L jars, and C$_2$H$_2$ added to 10% (v/v). Gas samples were taken 10 and 40 min later, and C$_2$H$_2$ analyzed by GC.

**RESULTS AND DISCUSSION**

**Greenhouse Plants.** Samples of xylem exudate taken weekly from plants between 40 and 75 DAP averaged 87%, 18%, and 28% of N as ureides for the plants grown with N$_2$, NO$_3$-, and NH$_4^+$, respectively. Amino acids averaged 11%, 40%, and 66% for the same samples with NO$_3$- comprising 2%, 4%, and 5% of the xylary N. Ureides were lowest and amino acids highest during the middle of the collection period. This agrees with other reports that the form of N in transport compounds of greenhouse-grown soybeans depends on the form in which N is supplied (15) and that most of the ureides are produced in the nodules (8, 24).

The high amino acid content of xylem sap taken from NO$_3$-grown plants supports reports that some NO$_3$- reduction takes place in soybean roots (15, 22). The xylem sap showed enrichment of $^{15}$N in the reduced fraction 1.5 h after beginning feeding with $^{15}$NO$_3$- (Table I). This enrichment was higher than in the phloem sap and was not therefore recycled N.

The N distribution into plant parts was similar among treatments. From nine Kjeldahl determinations between 41 and 64 DAP, leaves averaged 53.8 ± 0.5% (se), 50.5 ± 2.2%, and 53.7 ± 0.7% of the plant N for N$_2$, NO$_3$-, and NH$_4^+$, grown plants, respectively. The stems averaged 12.0 ± 0.9%, 9.3 ± 0.7%, and 11.8 ± 0.8% of the plant N for the same period and treatments. This means that the distribution of N above and below ground was similar for the treatments. However, the N$_2$-grown plants had 39% of the below ground N in the nodules. The per cent N in the roots of both N$_2$ (1.56 ± 0.05% [se]) and NO$_3$- (1.53 ± 0.05%) grown plants did not differ though the roots were smaller on the N$_2$-grown plants. Root growth by plants relying on N$_2$ fixation may be restricted (as suggested previously for Lupinus albus [18]), because of their increased requirement for photosynthate (23).

<table>
<thead>
<tr>
<th>Collection Location</th>
<th>Enrichment of $^{15}$N from Following Sources</th>
<th>%</th>
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<tbody>
<tr>
<td>Xylem sap (total)</td>
<td>$^{15}$N$_2$</td>
<td>$^{15}$NO$_3$-</td>
</tr>
<tr>
<td>27.0</td>
<td>15.1</td>
<td>15.0</td>
</tr>
<tr>
<td>Xylem sap (acids + neutrals)</td>
<td>31.9</td>
<td>28.9</td>
</tr>
<tr>
<td>Xylem sap (basics)</td>
<td>8.4</td>
<td>11.2</td>
</tr>
<tr>
<td>Petiole phloem sap</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Soluble stem N</td>
<td>0.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Soluble leaf N</td>
<td>0.4</td>
<td>2.0</td>
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</table>

* Primarily ureides.

**Table I. $^{15}$N Enrichment of the Transport Fluids and Plant Parts after Feeding $^{15}$N-Labeled Substrates to the Roots of 54-Day-Old Greenhouse-Grown Soybeans**

Each sample consisted of the bulked material from four plants. (Enrichment values are all corrected to 100% enrichment in the substrate). The tissue samples were extracted with 50 mm phosphate buffer. Feeding began 1.5 h prior to collection and continued during the 1-h collection.

**Table II. Distribution of $^{15}$N among Shoot Parts of Glasshouse-Grown Soybeans Fed Different $^{15}$N Sources to the Roots for 1.5 Hours Immediately Prior to Harvest**

<table>
<thead>
<tr>
<th>$^{15}$N Source</th>
<th>Plant Age</th>
<th>Stem</th>
<th>Leaflets</th>
<th>Pods</th>
<th>Axillaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{15}$N$_2$</td>
<td>54</td>
<td>40.6 ± 1.2</td>
<td>58.6 ± 7.0</td>
<td>0.39 ± 0.09</td>
<td>0.56 ± 0.28</td>
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<td>64</td>
<td>53.8</td>
<td>44.6</td>
<td>0.96</td>
<td>0.61</td>
</tr>
<tr>
<td>$^{15}$NO$_3$-</td>
<td>54</td>
<td>15.5 ± 2.5</td>
<td>82.9 ± 3.4</td>
<td>0.92 ± 0.25</td>
<td>0.73 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>15.3</td>
<td>84.2</td>
<td>0.31</td>
<td>0.18</td>
</tr>
<tr>
<td>$^{15}$NH$_4^+$</td>
<td>54</td>
<td>41.3 ± 5.1</td>
<td>56.8 ± 1.9</td>
<td>1.4 ± 0.60</td>
<td>0.55 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>42.6</td>
<td>56.7</td>
<td>0.38</td>
<td>0.25</td>
</tr>
</tbody>
</table>

The initial distribution of $^{15}$N was very different in plants grown on NO$_3$- compared to those grown on either N$_2$ or NH$_4^+$ (Table II). A considerable accumulation in the stem occurred only on the latter two forms of N. The similarity of relative enrichments of the phloem sap, however, indicated a similar proportion of the incorporated N was transferred from the xylem to phloem in all cases in spite of differences in the N distribution and form of the supplied N (Table I).

In spite of the different initial distributions of N shown by the short term $^{15}$N feeding experiment (Table II), a similar final partitioning was achieved for the three N sources when the $^{15}$N was traced for 2 weeks after feeding (Fig. I). For example, leaves and roots of NO$_3$-grown plants lost considerable $^{15}$N with time whereas stems increased in $^{15}$N content. This process was reversed for N$_2$-grown plants.

A number of factors were examined to determine how these differences in redistribution could have arisen. Apparently, composition of the phloem sap was independent of the source of N (Table III) with the ureides comprising a substantial, but not dominant, proportion in all cases. Three duplicated collections between 54 and 74 d of age for leaflets 2 to 6 contained 29 ± 10% (se), 32 ± 9%, and 32 ± 8% of the phloem N in ureides for NH$_4^+$, NO$_3$-, and N$_2$-grown plants, respectively. This occurred despite up to 5-fold differences in percentage of N as ureides in the xylem over this period.
FIG. 1. Distribution of $^{15}$N in plant parts 1, 7, and 14 d after feeding $^{15}$N as $N_{2}$ (A), $NO_{3}^{-}$ (B), or $NH_{4}^{+}$ (C). The parts 1 (root), 2 (nodules), 3 (stems), 4 (leaflets), 5 (axils), and 6 (pods and seeds) were analyzed as described in the text.

Table III. Phloem Composition of Leaflets 4 to 8 on 78-Day-Old Plants

Collections were made in EDTA solutions for 2 h after cutting the petiole. Each sample was four leaves from two plants, measured in triplicate. Each result is the mean ± SE of four samples.

<table>
<thead>
<tr>
<th>Phloem Constituent</th>
<th>N Source for Growth</th>
<th>NH$_4^+$</th>
<th>NO$_3^-$</th>
<th>$N_{2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars, $\mu$g leaf$^{-1}$ min$^{-1}$</td>
<td>1.60 ± 0.28</td>
<td>0.92 ± 0.16</td>
<td>0.93 ± 0.37</td>
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<tr>
<td>Amino acids, nmol leaf$^{-1}$ min$^{-1}$</td>
<td>1.02 ± 0.34</td>
<td>1.17 ± 0.35</td>
<td>1.16 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>Ureides, nmol leaf$^{-1}$ min$^{-1}$</td>
<td>0.38 ± 0.14</td>
<td>0.26 ± 0.04</td>
<td>0.27 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

Field experiments. Flowering began 7 weeks after planting and by 13 weeks the plants averaged 65% podfill. The final harvest data for the plants are given in Table IV (roots and fallen leaflets and petioles not included). Figure 2 shows the nitrogenase ($C_{2}H_{2}$) activity from 5 to 13 weeks after planting. For clarity, results from the middle level of N availability (20 kg N ha$^{-1}$) are omitted; they lie between the 0 and 100 kg N ha$^{-1}$ values. The decreased nitrogenase activity at week 7 could be a result of dry weather between weeks 4 and 7 (19).

Figure 3A shows the per cent N as ureides in xylem of nodulated soybeans. Again, data for 20 kg N ha$^{-1}$ are omitted. The average values of the per cent N as ureides in xylem for the entire collection period are given in Table IV. There is a close similarity during growth in the patterns of nitrogenase activity (Fig. 2) and the per cent N as ureides in xylem (Fig. 3A). When nitrogenase activity was maximum (weeks 9–11), the regression of acetylene reduction activity on per cent N as ureides gave a strong correlation ($r = 0.93$).

The relationship of xylem ureides to $N_{2}$ fixation observed in greenhouse-grown soybeans (14, 15) is therefore confirmed with field-grown soybeans. The results are similar to those obtained by analysis of ureides in shoot tissue; during early podfill, there is a strong correlation between the concentration of ureides in shoot and the acetylene reduction activity of roots (20).

Figure 3B shows the ureide contribution to xylem N in non-nodding plants. It is apparent that ureides in xylem are not necessarily linked to $N_{2}$ fixation. The lack of adequate levels of N in the soil (resulting in up to an 87% reduction in seed yield; Table IV) also leads to an increase in ureide percentage in the non-nodding plants (Fig. 3B). Non-nodding plants under N stress often exceeded some of the nodulated treatments for percentage of ureides in the xylem. Table IV shows that, averaged over the entire collection period, the non-nodding plants without added N equaled (31.8%) the xylary ureide percentage of the nodulated plants given the...
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19.1

4.0

54.0

31.8

24.4

31.8

24.4

29.6

45.4

31.8

15.8

9.0

+*, nodulated plants; -, non-nodulated plants.

Values are means of three replicates ± st of samples containing 10 plants. Values without st had the 30 plants bulked before analysis. Standard errors varied between 3 and 25% of the mean N value and averaged 13% of the mean N value.

(Total N nod plants - total N non-nod plant)/(total N non plant) × 100.

Harvest index is the total plant N that is in the seed, but does not include N loss in fallen petioles and leaflets or in roots and nodules.

Per cent ureides is the average percentage for the entire period of collection, weighing each week's collection equally.

highest level of added NO3- . This was in spite of substantial acetylene reduction activity in the latter (Fig. 2). Thus, the linkage between percentage ureides in the xylem sap and acetylene reduction was not independent of other factors affecting the plant (Fig. 4). We have shown (20) that source-sink manipulations do not always change nitrogenase (C2H2) activity and ureide content of shoot tissue in the same way.

The proportion of xyleary N as ureides depended on three factors: (a) the availability of soil N; (b) the production of ureides

by the roots; (c) the production of ureides by the nodules. The calculated export of ureides of non-nod roots appeared to be a constant factor among treatments. Between weeks 5 and 9, it averaged 1.50, 1.61, and 1.21 mg ureide N plant-1 d-1 at levels of applied NO3- of 0, 20, and 100 kg N/ha. (The values are determined as the appropriate N increments multiplied by the corresponding percentage ureides in the xylem sap [Fig. 3].) The export rate in non-nods was, therefore, low compared to nodulated plants (which reached up to 10.3 mg ureide N plant-1 d-1 for the most actively fixing plants). Nevertheless, it was sufficient to produce a high ureide percentage in the sap of N-deficient plants.

In spite of this great range in estimated reliance on fixation and percentage N as ureides in the xylem for nodulated plants, the per cent N in the seed at harvest showed no significant

<table>
<thead>
<tr>
<th>N Added</th>
<th>N in Seed</th>
<th>Seed N</th>
<th>Pod N</th>
<th>Stem N</th>
<th>N from Fixation</th>
<th>N Harvest Index</th>
<th>N as Ureides in Xylem</th>
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<tbody>
<tr>
<td>kg/ha</td>
<td>%</td>
<td>g/plant</td>
<td>%</td>
<td>%</td>
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<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>7.32 ± 0.08</td>
<td>0.82 ± 0.16b</td>
<td>0.038 ± 0.006b</td>
<td>0.026 ± 0.004b</td>
<td>82.9a</td>
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<td>+</td>
<td>0</td>
<td>100</td>
<td>7.36</td>
<td>1.007</td>
<td>0.048</td>
<td>0.041</td>
<td>53.7</td>
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<td>-</td>
<td>0</td>
<td>0</td>
<td>4.61 ± 0.09</td>
<td>0.135 ± 0.017</td>
<td>0.005 ± 0.001</td>
<td>0.011 ± 0.003</td>
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<tr>
<td>+</td>
<td>20</td>
<td>0</td>
<td>7.16 ± 0.08</td>
<td>0.78 ± 0.06</td>
<td>0.034 ± 0.003</td>
<td>0.031 ± 0.002</td>
<td>66.5</td>
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<td>-</td>
<td>100</td>
<td>0</td>
<td>5.30 ± 0.10</td>
<td>0.77 ± 0.10</td>
<td>0.035 ± 0.005</td>
<td>0.045 ± 0.006</td>
<td>59.4</td>
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<tr>
<td>+</td>
<td>100</td>
<td>0</td>
<td>7.30</td>
<td>0.89</td>
<td>0.048</td>
<td>0.049</td>
<td>44.6</td>
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<td>-</td>
<td>100</td>
<td>0</td>
<td>5.25 ± 0.07</td>
<td>0.31 ± 0.01</td>
<td>0.016 ± 0.001</td>
<td>0.200 ± 0.002</td>
<td>0</td>
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<tr>
<td>+</td>
<td>100</td>
<td>6.12</td>
<td>0.50</td>
<td>0.026</td>
<td>0.021</td>
<td>0.914</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Table IV. Harvest Data for all Treatments on Field-Grown Soybeans

Fig. 4. Relationship between xylary ureide content and acetylene reduction in field-grown soybean plants. Solid symbols represent all samples taken between weeks 9 and 11 after planting. Open circles (O) represent all other times for the same treatments from weeks 5 to 13. (O). Non-nodulated plants given 0, 20, 40, 100, or 200 kg N/ha as KNO3 at the same times as for the nodulated plants. (O). Nodulated plants given 0, 10, or 50 kg N/ha at weeks 1 and 3. (A), nodulated plants given an additional 20 or 100 kg N/ha at week 8.

Fig. 5. Ureide content of phloem sap collected from petioles 4 and 5 of field-grown soybean plants. Nodulated (O) and non-nodulated (A) plants were used, either untreated or fed 50 kg N/ha as KNO3 at weeks 1 and 3 (O,A). Phloem sap was the exudate from cut petioles into a 20 mm (pH 7.0) EDTA solution. Collection was in the dark for 2 h after cutting.
difference between NO₃⁻ treatments, except when small, late additions of NO₃⁻ inhibited fixation without supplying an adequate alternate source of N. In this situation the per cent N in the seed was reduced to a level similar to that of non-nod plants, which produced similar yields (Table IV).

Total yield was also unaffected by the proportion of N reaching the shoots as ureides. For nodulated plants, there was no significant chage in yield for plants receiving from 31.8% to 69.2% of their N as ureides (Table IV). Yield and per cent N in the seed seemed to be a consequence of the total N incorporated by the plant, but not its source.

The distribution of N between the above ground parts was also constant, in agreement with the N harvest index (Table IV) for nodulating (90 ± 1.1 (se)) against non-nod (90.7 ± 1.1) plants between high (91.3 ± 0.8) and low/medium (89.9 ± 1.3) fertilizer treatments. This was in spite of average differences in the percentage of ureides in the xylem of 2.4-fold and extreme differences of up to 8-fold. This shows that the form in which N reached the shoot did not affect its subsequent redistribution to the seed.

The partitioning of N during growth agreed with the results for greenhouse-grown plants. Table IV shows that this partitioning was independent of the reliance of the plant on fixation and also independent of the amount of N available to the plant. For example, the mean percentage of the plant N in the stem between weeks 5 and 13 was 20.0 ± 2.3 (se), 21.4 ± 2.5, 21.9 ± 1.6, and 25.8 ± 2.3 for non-nod and nodulated plants without supplemental N followed by the values for plants with 100 kg N/ha added (Table IV).

Figure 5 gives values for phloem ureides for the same four treatments given in Figure 2. The wide range of xylary ureides encompassed by these treatments (Table IV) apparently had no effect on the phloem ureide level. This was true not only for petiole phloem sap but also for that collected from the fruit tip. Analyses of petiole and fruit tip phloem sap collected at node 7 between 9 and 11 weeks gave 0.62 ± 0.10 (6), 0.55 ± 0.10 (6), (fruit tip, nod, and non-nod), and 0.31 ± 0.05 (8), (petiole, nod, and non-nod) nmol of ureides per µg sugars in the phloem (values are means ± SE with number of replicates in parentheses).

The amino content of the phloem sap was more variable; the averages of six to eight samples taken between weeks 5 and 9 from nodes 4 and 5 were 0.84 ± 0.15 (se), 0.81 ± 0.12, and 1.59 ± 0.40 mmol amino acids/µg sugar for 0, 20, and 100 kg/ha treated nodulated plants. For the corresponding non-nodulated plants, values was 0.81 ± 0.07, 0.70 ± 0.18, and 1.06 ± 0.31.

That the phloem composition is kept stable is, therefore, indicated both in greenhouse- and field-grown plants. The possibility of stem loading of N compounds (found previously in L. albus [18]) is suggested for ureides independent of the xylary ureide level. Evidence supporting this is presented elsewhere (13).

Our results confirm the relationship between ureides in xylem and N₂ fixation for greenhouse-grown soybeans (14, 15). However, the situation in the field was more complicated. A single measure of percentage N as ureides did not always give a good estimate of fixation. It may therefore be premature to use ureide content as a measure of the amount of N fixed by crops (9, 10).

Under all conditions studied, the xylem contained a substantial quantity of ureides recently produced from newly incorporated N (Table II) by non-nod roots. This suggests a situation similar to L. albus (16) in which different parts of the plant use different xylary N sources for growth. The reduced N in the xylem fluid of plants growing on NO₃⁻ may represent a specific source of N for stem growth as it was initially more strongly removed by the stem.

Why the nodules should produce ureides as a major source of xylary N while the root produces only small amounts is not clear. Other studies (27) have indicated that xylary composition of reduced N does not always depend on the source of N (either N₂ or NO₃⁻) in legumes. To compensate for these differences in the xylem sap, major metabolic changes must take place in the soybean shoot. To clarify these metabolic alterations the inputs, outputs, and accumulations of ureides and other metabolites must be measured throughout the life cycle for individual portions of the soybean plant (13).

Ultimately, the plant can compensate for changes in N source, with the result that per cent N in the seed and yield depend only on the total amount of N incorporated by the entire plant. Indeed, distribution of N within the plant, and N harvest index depend on neither the form nor the amount of supplied N. The composition of the seeds is maintained by the phloem sap which is the major source of seed N (18) and which had a constant composition under all the conditions we tested. Such a phenomenon may be common in legumes. How the factors involved in secondary distribution are modified to compensate for the changes in xylary composition are still unknown.

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